CD studies gave mirro-image curves to those described for the 1S,3R and 1R,3R compounds.

Acknowledgment. This work was supported by a grant from the Medical Research Council of Canada to Professor H. McLennan.

Registry No. 1, 62896-96-2; 2a, 13012-38-9; 2b, 71850-06-3; 3a, 112965-89-8; 3b, 112965-97-4; 3c, 112965-98-9; 3d, 112965-99-8; 4a, 111900-32-4; 4b, 111900-33-5; 4c, 89253-38-3; 4d, 111900-31-3.

$N,N$-Di-$n$-propylserotonin: Binding at Serotonin Binding Sites and a Comparison with 8-Hydroxy-2-(di-$n$-propylamino)tetralin

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8-Hydroxy-2-(di-$n$-propylamino)tetralin (8-OH-DPAT) is a serotonergic agonist with high affinity and selectivity for a particular population of central serotonin (5-HT) binding sites (i.e., 5-HT1A sites). Because the selectivity of 8-OH-DPAT may be due to the terminal amine substituents, the di-$n$-propyl analogue of 5-HT (i.e., 4) and of 5-methoxytryptamine (i.e., 5) were prepared and compared with 8-OH-DPAT with respect to their binding profile. Unlike 8-OH-DPAT, neither compound 4 nor 5 displays selectivity for 5-HT1A vs 5-HT3 sites. Consistent with these results, stimulus generalization occurs with 5 both in rats trained to discriminate 8-OH-DPAT from saline and in rats trained to discriminate the 5-HT3 agonist DOM from saline. The results of these study suggest that it is not the $N,N$-dipropyl groups that account for selectivity, but, rather, it is some feature associated with the pyrrole portion of the indolylalkanamines that is important.

Several different populations of central serotonin (5-hydroxytryptamine; 5-HT) binding sites have been identified in mammalian brain; these include 5-HT1A, 5-HT1B, and 5-HT2 sites.1 There is now evidence to suggest that members of a new class of anxiolytic agents (i.e., second generation anxiolytics) act as 5-HT1A agonists and that both thermoregulation and appetite control may also involve a 5-HT1A mechanism (see ref 1 for a review). These findings have focused considerable attention on this particular population of sites. The most potent and selective 5-HT1A agonist is 8-hydroxy-2-(di-$n$-propylamino)tetralin (8-OH-DPAT; 6);1-3 8-OH-DPAT binds at 5-HT1A sites with high affinity ($K_i = ca. 2 nM$), and [3H]-8-OH-DPAT is now commonly used to label these sites.1 An early report by Hoyer and co-workers4 showed that the di-$n$-propyl portion of 8-OH-DPAT makes a significant (>50-fold) contribution to its affinity for 5-HT1A sites. The affinity of 5-HT (1) for 5-HT1A sites is essentially identical with that of 8-OH-DPAT; however, 5-HT does not enjoy the selectivity displayed by 8-OH-DPAT. Arvidsson and co-workers5 demonstrated that the two n-propyl groups of 8-OH-DPAT are necessary for optimal activity in a biochemical measure of serotonergic activity (i.e., receptor-mediated feedback inhibition of 5-hydroxytryptophan accumulation). Aminotetralin analogues with alkyl groups smaller than n-propyl are less active whereas those with larger substituents are essentially inactive.5 They have also argued that 5-HT and 8-OH-DPAT share common aromatic and terminal amine sites in their binding to serotonin receptors.5 If this is the case, it may be possible to enhance the affinity and/or selectivity of 5-HT for 5-HT1A sites by incorporating the two n-propyl substituents onto its terminal amine. Consequently, $N,N$-di-$n$-propyl-2-[(5-hydroxyindol-3-yl)amino]ethane (i.e., $N,N$-di-$n$-propylserotonin; DiPS) (4) and its O-methyl ether, 5, were synthesized and evaluated.

Chemistry. Compound 4 has been previously mentioned in the literature,6-8 however, details of its synthesis

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the blood-brain barrier with difficulty, and due to the obtained with minal amine (in this case with the larger propyl groups) However, the effect of the dialkyl substituents on 5-HTlB groups of rats that had been previously trained to dis- hydroxyindole analogues (e.g., 5-HT, bufotenine) penetrate limited supply of DiPS. In these studies, doses of DPAT are not well tolerated at 5-HTlB sites. The affinity of 8-OH- Apparently, dialkyl substitution on the terminal amine is difference in the affinity of for (agonist-labeled) 5-HT sites. The selectivity of its at ~-HT,A and 5-HT2 sites, there is nearly a 50-fold dif- ference in the 5-HT agonists display a higher affinity for 0-methyl ether of 5-HT (i.e., 5-OMeT, respectively. Binding data for DOM are included for comparison. O-methyl ether of 5-HT (i.e., and for DOM (8, R = CH3) are also included for comparative purposes. The reason that two different radioligands were used to label 5-HT2 sites is that 5-HT agonists display a higher affinity for agonist-labeled (i.e., [3H]DOB-labeled) sites than for an- tagonist-labeled (i.e., [3H]ketanserin-labeled sites). 5-HT displays less than a 5-fold difference in affinity (Kj) for (agonist-labeled) 5-HT sites. The selectivity of its O-methyl ether 2 is essentially no different from that of 5-HT. Whereas the affinity of 3 is similar to that of 5-HT at 5-HT1A and 5-HT3 sites, there is nearly a 50-fold dif- ference in the affinity of 3 for 5-HT1A vs 5-HT3B sites. Apparently, dialkyl substitution on the terminal amine is not well tolerated at 5-HT1B sites. The affinity of 8-OH- DPAT (6) and its O-methyl ether 7 for 5-HT1A sites is also similar to that of 5-HT. Again, substitution on the terminal amine (in this case with the larger propyl groups) results in a very low affinity for 5-HT1B sites. As already demonstrated by others, 8-OH-DPAT is selective for 5- HT1A sites relative to 5-HT3 sites; similar results were obtained with 7 (Table I). DiPS (4) and its ether, 5, bind at 5-HT1A sites and 5-HT3 sites with high affinity but with little to no selectivity. However, the effect of the dialkyl substituents on 5-HT1B binding is still evident; the affinity of 4 and 5 for 5-HT1B sites is intermediate between that of 3 and 8-OH-DPAT (6) (Table I).

**Drug Discrimination Studies.** Because certain 5-hydroxyindole analogues (e.g., 5-HT, bufotenine) penetrate the blood–brain barrier with difficulty, and due to the limited supply of DiPS (4), compound 5 (and not 4) was selected for evaluation in the drug-discrimination paradigm. In these studies, doses of 5 were administered to groups of rats that had been previously trained to discriminate either 1.0 mg/kg of DOM or 0.2 mg of 8-OH-DPAT from saline. Both the DOM stimulus and the 8-OH-DPAT stimulus generalized to 5 (Table II). In both cases, however, response rates were reduced by greater than 50% (as compared to control values) at those doses where stimulus generalization occurred.

**Discussion**

As shown in Table I, DiPS (4) and its O-methyl ether 5 bind at 5-HT1A and 5-HT3 sites with very high affinity. Serotonin antagonists tend to show little difference in their affinity for 5-HT2 sites regardless of whether [3H]ketanserin or [3H]DOB is employed as the radioligand. Serotonin agonists, however, display a significantly higher affinity for [3H]DOB-labeled sites than for [3H]ketanserin-labeled sites. Thus, the data in Table I would suggest that 4 and 5 are serotonin agonists. Agonist activity can also be examined in drug-discrimination studies with tests of stimulus generalization. In such tests, the DOM stimulus has been shown to generalize to agents with high affinity for 5-HT2 sites, and the 8-OH-DPAT stimulus generalizes to agents with high affinity for 5-HT1A sites. In addition, animals trained to a site-selective agent do not normally recognize agents that are selective for a different site; for example, the DOM stimulus does not generalize to 8-OH-DPAT and visa versa. As shown in Table II, both the DOM stimulus and the 8-OH-DPAT stimulus generalize to O-Me-DiPS (6), suggesting that the agent, consistent with its binding properties, can produce both types of stimulus effects.

On the basis of the findings of Hoyer and co-workers, it was anticipated that DiPS (4) would possess an affinity for 5-HT1A sites greater than that of 5-HT; some selectivity for 5-HT1A sites might also have been expected. This was not found to be the case (Table I). Thus, although the propyl groups may contribute to the affinity of 8-OH-DPAT for 5-HT1A sites, those of DiPS (4) and 5 certainly have little effect on 5-HT1A-site affinity when compared to 5-HT (1) and 5-OMeT (2). 8-OH-DPAT is known to be selective for 5-HT1A sites relative to 5-HT2 sites. The data in Table I for 8-OH-DPAT (6) and 8-O-Me-DiPS (7) are consistent with these findings regardless of which radioligand is used to label the 5-HT2 sites. But again, the lack of selectivity of DiPS (4) and O-Me-DiPS (5) for 5-HT1A vs 5-HT2 sites, as compared with 5-HT (1) and 5-OMeT (2), argue against a role for the propyl groups in imparting 5-HT1A vs 5-HT2 selectivity. The propyl groups of compounds 4–7 do, however, seem to contribute to selectivity in that, relative to 5-HT, these agents bind at 5-HT1A sites with a significantly higher affinity than they display for 5-HT1B sites. How can the selectivity of 8-OH-DPAT (6) and 8-O-Me-DiPS (7), relative to that of DiPS (4) and O-Me-DiPS (5), for 5-HT1A sites vs 5-HT2 sites be accounted for? There are several potential explanations. The amino- tetralines (i.e., 6 and 7) and the indolealkylamines (i.e., 4 and 5) may bind in a different fashion; in this case, it may be argued that different conformations or terminal amine sites are involved. However, certain ergolines (which contain both amiotetralin and indolylalkaneamine fragments within their tetracyclic framework) display very high affinity and little to no selectivity for 5-HT1A vs 5-HT2 sites. For example, (+)-lysergic acid diethylamide binds both at 5-HT1A sites (Ki = 0.4 nM) and at [3H]DOB-labeled 5-HT2 sites (Ki = 0.5 nM). This would suggest that a common conformation, such as that found in the ergolines, is important.

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Another possible explanation for the observed selectivity is that the pyrrole portion, or some feature (e.g. electron density) associated with the pyrrole portion, of the indolealkylamines is important for affinity at 5-HT₂ sites but not for affinity at 5-HT₁₂ sites. Thus, 8-OH-DPAT (6) and 8-OMe-DPAT (7), which lack this feature, display a low affinity for 5-HT₂ sites. There are several other lines of evidence to support this argument: (a) simple tryptamine derivatives generally display a high affinity but low selectivity for 5-HT₂ sites,¹⁴ (b) other 5-HT₁A-selective agonists such as buspirone and gepirone lack a pyrrole ring, and (c) fusion of a pyrrole ring to the aminotetralin counterpart of DOM (i.e., DOB; 8, R = Br). We have proposed that these types of compounds interact with 5-HT₂ sites in such a manner that the meta methoxy group might interact with that portion of the receptor that normally accommodates the pyrrole portion of the indolealkylamines.¹⁵ DOB displays a very high affinity and selectivity for 5-HT₁A sites but, at least initially, the low affinity of these agents for 5-HT₁B sites but, at least initially, the low affinity of these agents for 5-HT₁B sites is not related to the absence or presence of a pyrrole-ring feature, which appears to be important for binding at 5-HT₂ sites.

Experimental Section

Synthesis. Proton magnetic resonance spectra were obtained with a JEOL FX90Q spectrometer with tetramethylsilane as an internal standard; infrared spectra were recorded with a Perkin-Elmer 257 spectrophotometer. Spectral data are consistent with the assigned structures. Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected.

Table II. Results of Stimulus Generalization Study

<table>
<thead>
<tr>
<th></th>
<th>dose⁴</th>
<th>N⁵</th>
<th>drug-appropriate responding, % (±SEM)</th>
<th>resp/min (±SEM)</th>
<th>ED₅₀, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Me-DIPS (5)</td>
<td>0.6</td>
<td>5/5</td>
<td>21 (5)</td>
<td>10.2 (1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>5/5</td>
<td>49 (18)</td>
<td>12.5 (2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>4/5</td>
<td>51 (12)</td>
<td>12.0 (1.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4/5</td>
<td>74 (12)</td>
<td>10.3 (2.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>3/5</td>
<td>91 (5)</td>
<td>5.2 (2.0)</td>
<td></td>
</tr>
<tr>
<td>5-OMe-DMT (3)</td>
<td>1.0</td>
<td>5/5</td>
<td>92 (4)</td>
<td>12.8 (1.8)</td>
<td>1.68 (0.75-3.76)</td>
</tr>
<tr>
<td>DOM saline (1.0 mL/kg)</td>
<td>1.0</td>
<td>5/5</td>
<td>12 (3)</td>
<td>11.5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>O-Me-DiPS (5)</td>
<td>0.4</td>
<td>4/5</td>
<td>15 (10)</td>
<td>13.6 (2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>3/4</td>
<td>56 (12)</td>
<td>9.2 (2.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>4/7</td>
<td>56 (13)</td>
<td>13.6 (4.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4/5</td>
<td>67 (16)</td>
<td>5.6 (1.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>4/5</td>
<td>81 (8)</td>
<td>4.1 (0.8)</td>
<td>0.75 (0.49-1.18)</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>2/5</td>
<td>e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OMe-DPAT (7)</td>
<td>0.2</td>
<td>7/7</td>
<td>88 (6)</td>
<td>16.8 (2.3)</td>
<td>0.08⁴</td>
</tr>
<tr>
<td>8-OH-DPAT (6)</td>
<td>0.2</td>
<td>7/7</td>
<td>16 (4)</td>
<td>16.0 (2.4)</td>
<td></td>
</tr>
<tr>
<td>saline (1.0 mL/kg)</td>
<td>0.2</td>
<td>7/7</td>
<td>88 (6)</td>
<td>16.8 (2.3)</td>
<td>0.08⁴</td>
</tr>
</tbody>
</table>

⁴Dose in mg/kg. ⁵Number of animals responding/number receiving drug. ¹ED₅₀ followed by 95% confidence limits. ²ED₅₀ value previously published.¹³ ³No responding (i.e., disruption of behavior). ⁴ED₅₀ previously published.¹³

provided 50 mg (65%) of the title compound (as off-white prismatic crystals), mp 197–199 °C (lit. mp 204–205 °C).

Binding Studies. The radioligand binding assay was conducted in essentially the same manner as reported earlier.1,18 Following decapitation, the brains of male Sprague–Dawley rats (ca. 220 g) were removed and placed in 0.9% ice-cold saline and dissected over ice until the tissue was prepared. Tissues were stored in ice-cold saline for no longer than 1 h and, following blot drying and weighing, were prepared and frozen at −30 °C until used. Freshly dissected tissue was homogenized (Polytron setting 6 for 20 s) in 30 volumes of ice-cold buffer containing 30 mM Tris·HCl (pH 7.4 at 37 °C; pH 8.0 at 4 °C), 0.5 mM Na2EDTA, and 10 mM MgSO4, and centrifuged at 3000g for 15 min. The supernatant was discarded, and the pellet was resuspended and preincubated for 15 min at 37 °C. The pellet was washed twice by centrifugation and resuspension. The final assay buffer contained 50 mM Tris·HCl (pH 7.7), 10 μM pargyline, 0.1% ascorbate, 10 mM MgSO4, and 0.5 mM Na2EDTA. The agonist high-affinity state of the 5-HT2 receptor was labeled with 0.4 nM [3H]DOB (40 Ci/mmol; New England Nuclear) with 20 mg wet weight of tissue prepared from rat frontal cortex. Cinanserin (1 μM) was used to define nonspecific binding. 5-HT1B sites were also labeled with [3H]ketanserin (76 Ci/mmol; New England Nuclear) and 3 μg wet weight of rat frontal cortex tissue. The 5-HT1B receptor was labeled with 0.1 nM [3H]8-OH-DPAT (120 Ci/mmol; New England Nuclear) and 6 μg wet weight of rat hippocampal tissue. 8-OH-DPAT (1 μM) was used to determine nonspecific binding. The 5-HT1B receptor was labeled with 2 nM [3H]serotonin (23 Ci/mmol; New England Nuclear) and 8 μg wet weight of rat striatum. Serotonin (10 μM) was used to define nonspecific binding; 8-OH-DPAT (100 nM) and mesulergine (100 nM) were used to block 5-HT1A and 5-HT1C sites, respectively. Eleven concentrations of nonradioactive competing drugs were made fresh daily in assay buffer. Following incubation with membranes and radioligand at 37 °C for 15 min (5-HT2 assays) or for 30 min (5-HT1 assays), samples were rapidly filtered with glass-fiber filters and were washed with 10 μL of ice-cold 50 mM Tris·HCl buffer. Individual filters were inserted into vials and equilibrated with 5 mL of scintillation fluid for 6 h before counting at 45% efficiency in a Beckman 3801 counter. Results were analyzed with RSI (BBN Software).

Discrimination Studies. The present study used male Sprague–Dawley rats that had been previously trained17,19 to discriminate either 1.0 mg/kg of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) or 0.2 mg/kg of 8-hydroxy-2-(N,N-di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) from 0.9% saline. The studies were conducted with use of standard two-lever operant chambers (Coulbourn Instruments Model E10-10), a variable-interval 15-s schedule of reinforcement for food (sweetened milk) reward, and a 15-min pre-sessions injection interval. Details of the training and testing procedures have been previously reported.17,19 Briefly, in the tests of stimulus generalization (during which maintenance of the original DOM/saline or 8-OH-DPAT/saline discrimination was insured by continuation of training sessions throughout the studies), the animals were allowed 5.5 min to respond under extinction conditions and were then returned to their individual home cages. An odd number of training sessions (usually five, but never less than three) separated any two test sessions. During the test sessions, doses of the challenge drugs were administered by intraperitoneal injection in a random order to, routinely, groups of five animals. Once disruption of behavior occurred, only lower doses of the compound would be investigated. A 15-min pre-injection interval was used throughout. Stimulus generalization was said to have occurred when the animals made ≥80% of their total responses on the drug-appropriate (i.e., either DOM-appropriate or 8-OH-DPAT-appropriate) lever. Animals making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. Where stimulus generalization occurred, ED50 values (i.e., doses at which the animals would be expected to make approximately 50% of their responses on the drug-appropriate lever) were calculated from the dose–response data.

Acknowledgment. We express our appreciation to M. Seggel and N. Naiman for their assistance with certain synthetic aspects of this project.

Peptide Derivatives of Primaquine as Potential Antimalarial Agents

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Received August 28, 1987

Three peptide derivatives of primaquine were synthesized. The compounds were tested for radical curative antimalarial activity against Plasmodium cynomolgi in rhesus monkeys and blood schizonticidal antimalarial activity against Plasmodium berghei in mice. All three peptide derivatives showed activity against P. cynomolgi greater than that expected for the primaquine content of each prodrug. The toxicity of one of the peptide derivatives was less than that of primaquine in mice.

Malaria is caused by more than 50 different species of the protozoan Plasmodium.1 However, only four species attack humans: P. falciparum, P. vivax, P. malariae, and P. ovoide. If not treated properly, vivax malaria may subside spontaneously, only to recur at a later date. Primaquine (I) is the drug of choice for the radical cure of vivax malaria, or in combination with other antimalarial drugs such as chloroquine for prophylaxis. The major drawback of primaquine is its low therapeutic index. The drug is known to cause hemolytic lesions, particularly in patients deficient in glucose-6-phosphate dehydrogenase. Many analogues of I have been prepared, some less toxic than I.2,3 Recently, reduced toxicity was achieved by encapsulating primaquine in liposomes4 or by linking 1 to a macromolecular protein.5,6 Interestingly, Trouet and co-


